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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Stamler *et al.*		
SERIAL NUMBER:	10/066,320	EXAMINER:	Anish Gupta
FILING DATE:	January 31, 2002	ART UNIT:	1654
FOR:	Method for Determining Physiological Effects of Hemoglobin		

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF JONATHAN S. STAMLER UNDER 37 C.F.R. §1.132

I, Jonathan S. Stamler, of 101 Juniper Place, Chapel Hill, North Carolina, 27514, declare and state that:

1. I am a coinventor, together with Andrew J. Gow and David J. Singel, in the above-referenced patent application.

2. I received an M.D. from Mount Sinai School of Medicine in New York and then completed my medical residency and fellowship training in both cardiology and pulmonary medicine at Harvard Medical School and the Brigham and Women's Hospital. I hold the position of George Barth Geller Professor for Research in Cardiovascular Diseases, Professor of Medicine and Biochemistry, and an Associate Investigator of the Howard Hughes Medical Institute at the Duke University Medical Center in Durham, North. I have published numerous scientific articles in the field of nitric oxide and nitric oxide donors.

3. I have reviewed the Advisory Action mailed November 15, 2006 and the Final Office Action mailed June 27, 2006. I understand that claims 4-6 and 30-35 are rejected under 35 U.S.C. §112, first paragraph for failing to comply with the enablement requirement.

4. I disagree with the Examiner's assertion that the instant specification does not describe all the desired conditions to achieve SNOHb. I also disagree with the Examiner's assertion that the results of the instant application describe that the desired SNOHb is not obtained under the described experimental conditions (*e.g.*, Example 3) and that the one of ordinary skill in the art would be burdened with undue experimentation to determine the appropriate conditions for obtaining SNOHb rather than obtaining methemoglobin (metHb) or iron-nitrosylhemoglobin

5. I submit that the instant specification describes two conditions essential to modulate the interaction between NO and oxyhemoglobin. First, for storage of NO (either in non-donor form as iron nitrosylhemoglobin or in donor form as SNOHb), the R structure of hemoglobin must be maintained. The instant specification further describes that the R structure of hemoglobin can be maintained under low phosphate conditions (*i.e.*, less than 100 mM and preferably 10mM). Second, for the generation of NO bioactivity in donor-form as SNOHb, the redox chemistry of hemoglobin capability must be preserved to permit the transfer of NO from the heme Fe to cysteine on the β subunit. These two conditions are illustrated in Examples 3 and 4 and were part of a peer-reviewed scientific manuscript published in Proc Natl Acad Sci U S A. *See*, Appendix A.

6. I submit that the experiments in Example 3 were not designed to measure the yield of SNOHb under those conditions. Rather, the experiments described in Example 3 were designed to measure the yield of iron-nitrosylhemoglobin vs. metHb under low phosphate conditions (*i.e.*, less than 100 mM and preferably 10mM), plus or minus borate. Borate is a unique compound which inhibits cysteine NO-reactivity and prevents the transfer of NO from heme. Thus, the addition of borate to the low phosphate conditions is used to assess the second step described above; that is, the ability to form SNOHb following the transfer of NO from the heme Fe to cysteine on the β subunit.

   The results in Example 3 demonstrate that under low phosphate conditions (plus or minus borate) formation of iron-nitrosylhemoglobin is favored over metHb. These results demonstrate the first essential condition: that maintenance of the R structure of hemoglobin favors the storage of NO (either in non-donor form as iron

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nitrosylhemoglobin or in donor form as SNOHb). However, these measurements do not measure the difference between iron nitrosylhemoglobin or SNOHb under the low phosphate conditions (plus or minus borate). Had metHb, iron-nitrosylhemoglobin and SNOHb been analyzed simultaneous under the conditions of Example 3, metHb formation would have been favored under high phosphate conditions, iron-nitrosylhemoglobin formation would have been favored under low phosphate conditions plus borate and SNOHb formation would have been favored under low phosphate conditions in the absence of borate.

7. I submit that the experiments described in Example 4 were designed to measure the yield of SNOHb under low phosphate conditions in the absence of borate and the results of those experiments show that SNOHb and intraerythrocytic SNOHb are produced under those low phosphate conditions in the absence of borate. The results in Example 4 demonstrate that for the storage of NO in donor-form as SNOHb, the redox chemistry of hemoglobin capability must be preserved and the cysteine must display NO-reactivity to permit the transfer of NO from the heme Fe to cysteine on the β subunit.

8. I further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Jonathan S. Stamler

Signed this 17th day of January, 2007

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